

Electromyographic Record of Classical Conditioning of Eye Withdrawal in the Crab

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Abstract. Classical (Pavlovian) conditioning of the eye withdrawal reflex of the green crab, *Carcinus maenas*, was studied by recording electromyograms (EMGs) from the main abductor muscle of the eye (19a). The EMG record was a reliable indicator of the response, and it was always correlated with physical movement of the eye, whether evoked by the unconditioned stimulus (a puff of air to the eye), or by the conditioned stimulus (a mild vibration of the carapace). The EMG was used to study the acquisition of conditioned responses in animals with an immobilized eye. Six of eight experimental animals developed responses to the conditioned stimulus in a manner similar to that for animals with freely moving eyes; unpaired controls showed few responses. The results indicate that eye movement is not required for learning. Behavioral tests after conditioning and after the eyes had been freed supported this conclusion. The results exclude theories of classical conditioning of eye withdrawal that invoke a role for stimuli due to eye movement (such as a change in visual field).

Introduction

The eye withdrawal reflex of the crab is one of the simple invertebrate behaviors in which learning can be demonstrated (Abramson and Feinman, 1987; Abramson *et al.*, 1988; Abramson and Feinman, 1988; Appleton and Wilkens, 1990). Classical (Pavlovian) condition-

ing of the response is brought about by pairing a previously neutral stimulus (vibration of the carapace) with an aversive stimulus (an air-puff to one of the eyes). The air-puff [unconditioned stimulus (US)] invariably causes eye retraction. After several pairings of the US with the vibration [conditioned stimulus (CS)], eye retraction begins to appear during CS presentations. The responses can be recorded in several ways. In addition to direct observation, movement can be recorded by optical or capacitive methods (Sandeman, 1968; Forman and Brumbley, 1980; Miall and Hereward, 1988), or by the force generated during retraction (Erber and Sandeman, 1989; Appleton and Wilkens, 1990). Electromyograms (EMGs) are also easily recorded (Burrows and Horridge, 1968) and, in this report, we describe the use of EMGs recorded from the main abductor muscle of the eye (muscle 19a) as an indicator of the response. The method allows us to record responses in the restrained eye, and we use it to show that physical movement of the eye is not required for learning.

One of the virtues of this system is that some of the physiology has already been characterized (Burrows, 1967; Sandeman, 1967, 1969b) and, therefore, the neuronal substrate of conditioning may be accessible. Several features of eye withdrawal make it desirable for such an analysis. Retraction is mediated by only two motor neurons, one of which is identified and has a giant axon (Burrows, 1967; Sandeman, 1967, 1969a; Burrows and Horridge, 1968); the activity of this unit is the signal of greatest amplitude in the EMG recorded from muscle 19a. Studies of eye withdrawal have shown that there is no requirement for proprioceptive feedback; whether this is true under conditions where learning occurs is unknown. Although less well characterized, the sensory

Received 18 January 1990; accepted 22 March 1990.

Abbreviations: Electromyogram (EMG); conditioned stimulus (CS); unconditioned stimulus (US); conditioned response (CR); and unconditioned response (UR).

afferents are also known and are believed to make largely monosynaptic contacts with the motor neuron (Sandeman, 1969a, 1969b).

The role of eye movement also bears on long-standing problems in the psychology of learning. The eye withdrawal reflex can be trained in a signalled avoidance procedure in which US presentation can be avoided, if the eye is retracted during the CS, which acts as a "warning signal" (Abramson *et al.*, 1988). Acquisition of conditioned eye withdrawal in avoidance followed a time course similar to that for classical conditioning, suggesting that animals might not benefit from being able to control the contingencies of reinforcement. In other words, the animal might effectively have been in a classical conditioning experiment in which some USs were omitted. The paradox is that controls that were subjected to the same sequence of USs and omissions did poorly, whereas, if the contingency between eye withdrawal and absence of US were not important, they should have done as well as the experimentals. A similar result has been observed for vertebrates in some learning procedures (Moore and Gormezano, 1961; Gormezano, 1965; Woodward and Bitterman, 1973). One theory that has been proposed to explain these results is that animals are receiving a compound CS composed of the vibration *plus* the change in sensory input (such as visual field) that occurs as a consequence of the eye movement. The results reported here suggest that the consequences of the eye movement do not play a necessary role in classical conditioning, and therefore, that the theory cannot explain the similarity of classical conditioning and avoidance, at least in the crab eye withdrawal reflex.

Materials and Methods

The general experimental setup for classical conditioning has been described (Abramson and Feinman, 1988). The CS was a low amplitude 200 Hz vibration administered to the carapace via a needle attached to a loudspeaker. The US was a low intensity puff of air delivered to the eye to be conditioned. In the experiments described here, a 1-s presentation of the CS was followed immediately by a 0.1-s presentation of the US. In general, the eye was re-elevated after the retraction; in cases where this did not occur, the animal was gently tipped or one of the legs was moved to cause the eye to come back up. For recording myograms, a single hole was made, with the tip of a hypodermic needle, in the cuticle surrounding the eye, and two 50- μ wires were inserted into muscle 19a and attached to the cuticle with cyanoacrylate glue. Placement of electrodes was confirmed by dissection of formaldehyde-fixed samples. The insertion of the EMG electrodes had a sensitizing effect, and animals would respond to a level of vibration that was normally without effect. Thirty minutes after implanting the elec-

trodes, this sensitivity was sufficiently reduced so that there was no response to three or four successive stimuli. Scoring of conditioned responses in myographic records of animals with restrained eyes was done blind; a naive observer was instructed to score EMG patterns during the CS that resembled those seen during the US.

Results

Electromyographic measurement of acquisition

The first experiment demonstrated the feasibility of using the EMG record to follow conditioning. Four experimental animals and four controls had EMG electrodes implanted in muscle 19a of one eye; the eye moved freely after this manipulation. The experimentals were subjected to 50 paired presentations of stimuli as described in Materials and Methods; controls were given 50 presentations of unpaired stimuli. Panel A of Figure 1 shows EMG records of several trials for one of the experimental animals. The characteristic spiking pattern due to the activity of the fast retractor motor neuron of the optic nerve is reliably seen in response to presentation of the US. Slow tonic activity is also seen in some traces in panel A. These are due to the activity of a smaller neuron of the oculomotor nerve; the tonic firing of this unit correlates with the eye being held down (Sandeman, 1964; Burrows, 1967; Burrows and Horridge, 1968). Muscle 19a is more sparsely innervated by this neuron than by the larger retractor neuron, and the tonic activity is not seen in every preparation. After several trials, a pattern of spiking activity similar to that caused by the US is now evoked during the CS. This pattern in the CS or US was always correlated with observed retraction of the eye.

Two features of the EMG record were not obvious from simple observation of the gross behavior. First, as is evident in Figure 1, the conditioned responses (CRs), when they appear, are frequently more robust than the unconditioned response (UR). In addition, although not a feature of all sessions, the UR frequently showed habituation even as the CR developed (data not shown). This phenomenon has been studied more thoroughly by Appleton and Wilkens (1990). The pattern of acquisition seen in the present work is qualitatively similar to the acquisition of CRs as previously described (Abramson *et al.*, 1988; Abramson and Feinman, 1988). There were few, if any, spontaneous eye retractions (or bursts of phasic activity in the EMG record) during the intervals between stimuli presentation.

To assess the effect of the insertion of electrodes, the behavior was compared to that of a second group of four experimental and four unpaired control animals that had never had EMG wires implanted. Responses of the experimentals and the controls were tallied and the aver-

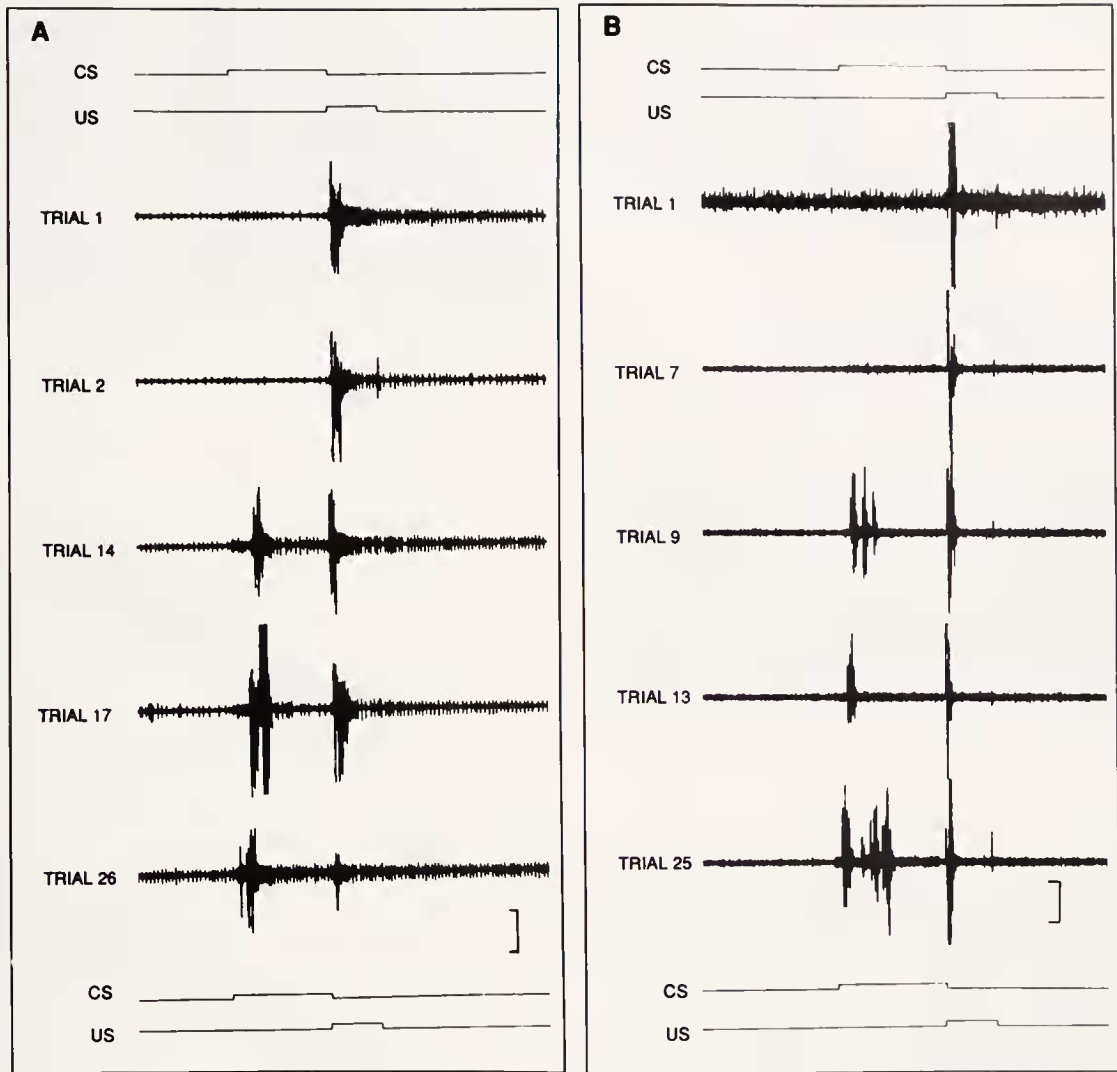


Figure 1. Electromyographic record of classical conditioning. EMGs were recorded from muscle 19a of the eye to be conditioned. A. Results for a typical animal with a freely moving eye. B. Results while the eye is physically restrained. Large amplitude spikes are due to activity of the fast phasic motor neuron of the optic nerve. Slow tonic activity evident in traces in panel A are due to a neuron of the oculomotor nerve which more sparsely innervates 19a and whose activity correlates with maintenance of the retracted state. The CS duration is 1 s. The vertical bar corresponds to 200 μ V except in TRIAL 1 of panel B where it represents 100 μ V. Animals were trained with paired presentation of CS and US (top and bottom traces). Animals in panel B had the eye temporarily immobilized with a rubber band.

age responses for each five-trial block were plotted (first panel of Fig. 2). The behavior of the two sets of animals, with and without EMG wires, is manifestly similar: the paired animals of each group showed an increase in the probability to respond reaching a plateau probability of 50–60%, whereas the corresponding unpaired groups showed a much lower tendency to respond (see below for statistical comparison). Thus, learning is fundamentally the same in animals with and without EMG electrodes; for qualitative comparisons to animals with restrained eyes, these two groups were pooled and considered as a

population of eight animals trained with freely moving eyes. However, there were some differences. First, Figure 2 shows that the EMG animals were sensitized, as indicated by their higher probability to respond at the outset of training (first 5-trial block). The mean probability of response for EMG animals in this period was 0.35 (SD 0.25) compared to 0.05 (SD 0.10) for unoperated animals. A second difference is the somewhat greater variability in the EMG animals. To see this difference we plotted individual animal data as a cumulative record, or running total, in Figure 3 (panels A and B). Usually

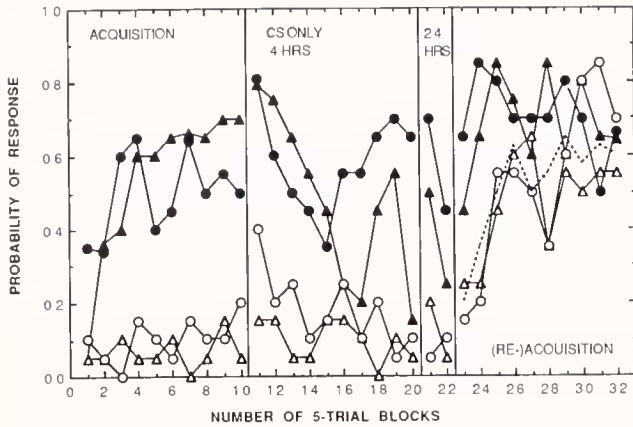


Figure 2. Effect of implanting EMG electrodes during acquisition on behavioral performance. Group data for behavior in ACQUISITION, RETENTION, and RE-ACQUISITION of classical conditioning. Data points are averages of four animals each. Filled symbols: animals receiving paired stimuli during ACQUISITION. Open symbols: animals receiving specifically unpaired stimuli during ACQUISITION. Two populations were used. Triangles: normal unoperated animals; Circles: animals with EMG wires implanted. In RE-ACQUISITION, dotted line is first day performance of the average of the (8) experimental animals and is included for comparison. Probability of response is calculated as the total number of responses per animal per five-trial block.

applied to operant conditioning experiments, a cumulative record is a good method for looking at trial-by-trial data. It is evident that, again, the groups are very similar, but inserting the EMG wires introduces variability in the pattern of response. In summary, the EMG record is a reliable method for following conditioning—the large differences between paired and unpaired groups are maintained—but the process of inserting electrodes may have a somewhat sensitizing effect on the CS responses.

Pattern of behavior after conditioning

As a second method of assessing the effect of training, we recorded a profile of behavioral responses after conditioning. For animals with EMG leads, wires were cut. All animals were returned to the home tank and then all (paired and unpaired controls) were tested for responses in three behavioral procedures. First, after 4 h, animals were given 50 CS-only presentations (second panel of Fig. 2). Then, after an additional 20 h, they were re-tested for responses to 10 CS-presentations (third panel of Fig. 2). Immediately after these 10 CS-only trials, animals were subjected to a second training session (last panel). During this second training period, the unpaired controls from the first day were given paired presentation of stimuli to determine whether this population was, in fact, capable of learning and whether there was an effect of the previous day's experience as controls. It is evident from

Figure 2 that: the paired group showed substantial retention after 4 h as measured by the CS-only responses, and that extinction is fairly rapid; unpaired controls showed few CRs; and in both cases there was a considerable variation among animals. There is also a rebound of the experimental's response to the conditioned stimulus after 24 h; the unpaired group, again, showed few responses. The last panel in Figure 2 indicates an enhanced re-acquisition of the task by the subjects that had been experimental on the first day; this is consistent with earlier re-

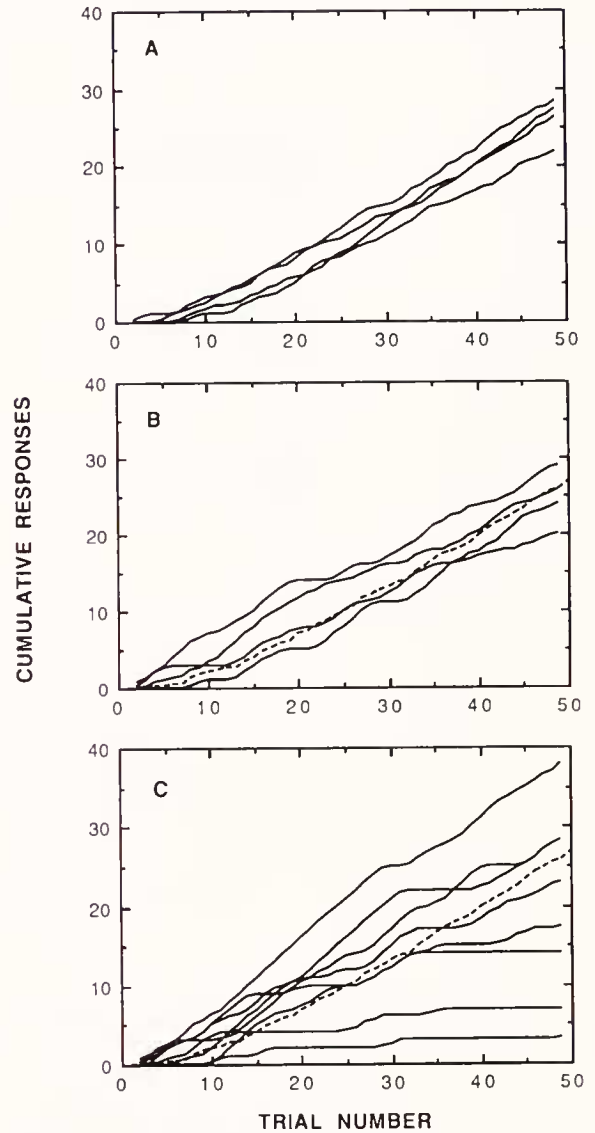


Figure 3. Cumulative record for acquisition of conditioned responses. Results are shown for all experimental (paired CS, US) subjects. A. Normal subjects, unoperated. B. Freely moving eyes with EMG electrodes implanted. C. Animals with EMG leads and conditioned eye immobilized. In B and C, the dotted line represents the average of the records for the four animals in A. Data were smoothed, for graphic clarity, by averaging over three trials at a time.

ports (Abramson and Feinman, 1988). Likewise, controls from day-one now showed a high probability to respond, indicating that there was nothing unusual about this group and that their performance was not repressed by their previous experience as unpaired controls, again consistent with original observations. Figure 2 shows that this day-two acquisition by controls has a very similar time dependence to the day-one acquisition by experimentals (dotted line), indicating that the controls were also not sensitized and had not fortuitously made a CS-US association. This general pattern of responses was similar for both groups: normal animals and those with EMG electrodes.

Electromyographic record of conditioning of a restrained eye

With the behavioral pattern of acquisition, retention, and re-acquisition as background, we next prepared 16 new animals with silver wire electrodes in the eye and now restrained one eye (to be conditioned) with rubber bands. Eight of these animals were subjected to the paired presentation of stimuli as above, while the other eight served as controls and were given specifically unpaired CS, US presentations. Conditioned responses were scored from the EMG record. Activity during CS presentations that resembled those during the US were considered conditioned responses. Figure 1B shows characteristic EMG patterns typical of these animals. Six of the eight experimental animals showed development of a conditioned EMG response in a manner similar to the groups with freely moving eyes. None of the unpaired controls showed the normal acquisition, although one animal gave several responses during the first few trials, presumably due to the sensitizing effect of the manipulations.

Panel C of Figure 3 shows the cumulative records for the eight animals in the experimental paired group. Some animals showed behavior clearly similar to that of animals whose eyes were not restrained (panels A and B), and some are actually sensitized compared to normals. Two animals made few responses, and one initially showed good acquisition but stopped responding at trial 32. Thus, six of the eight animals showed a pattern of responding similar to animals with freely moving eyes for more than 60% of the training session. Figure 5 shows that these six animals also gave more total responses than any unpaired animal in the experiment. Using these arbitrary criteria, we would say that six of the subjects were conditioned. There is also greater variability of individual animals with restrained eyes (first panel of Fig. 5).

The two experimental animals that did not learn (see above; Fig. 3A, B) did show small bursts of phasic activity during the CS presentations. In animals with freely

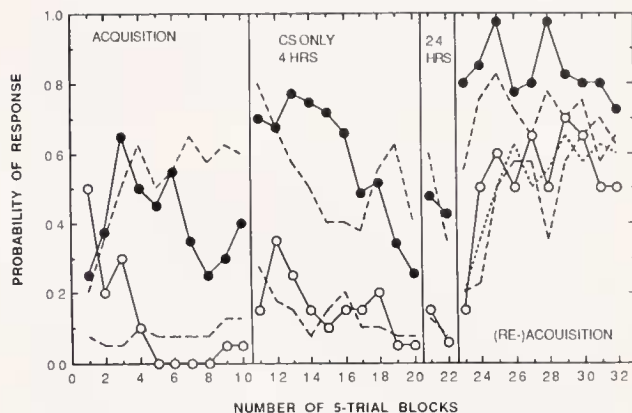


Figure 4. Effect on behavioral performance of immobilizing the eye during acquisition. Group data for animals trained with immobilized eyes compared to animals with freely moving eyes. Data points are averages of eight animals each. Filled symbols: experimentals; open symbols: unpaired controls. Broken line: average of corresponding data from Figure 2 [data from 4 normal and 4 freely moving eye with electrodes were pooled and averaged for each of the two groups (paired and unpaired)]. Data points in ACQUISITION are EMG responses; other data, retention and re-acquisition of classical conditioning, are recorded behaviorally. In RE-ACQUISITION, dotted line is first day performance of the average of experimental animals redrawn for comparison.

moving eyes, these would correlate with small twitches of the eye, but are not normally scored as full responses. This suggests that even the animals that did not meet the criterion of EMG responses that resembled those to the US may have acquired some association from the training. This idea was strengthened by their subsequent performance in the behavioral tests described below.

Behavioral tests after acquisition

After the acquisition trials, the eyes were freed, the EMG leads were cut, and the animals were returned to their home tanks. They were then tested, as were animals trained with freely moving eyes, for responses in the behavioral tests: retention after 4 h and after 24 h, and re-acquisition in a second training session. The results are shown in Figure 4, where they are compared to the averaged data for the two groups trained with freely moving eyes. When the qualitative behavior of the animals trained with restrained eyes is compared for retention and reacquisition to that for animals with freely moving eyes (Figs. 2, 4), similar profiles are found, although, as noted above, the response to CS-only presentations varies substantially. During re-acquisition, behavior of the animals trained with restrained eyes is remarkably like that for animals with moving eyes: all experimentals show enhanced probability of responding, and all controls now subjected to paired training behaved like day-one experimentals. This behavioral performance of the

experimentals suggests that learning took place during day-one acquisition even in the case of the two animals where an EMG response was not evident.

Summary of statistical analysis

The major conclusions bearing on acquisition were that the groups presented with paired stimuli showed an increased probability to respond to the CS when compared to controls, and that the effect of EMG electrodes was somewhat sensitizing in terms of individual performance, although there were no differences over the course of the training. These conclusions are supported by an analysis of variance conducted over the 10 five-trial blocks of acquisition. For normal unoperated animals, differences between paired and unpaired groups was significant, $F(1,60) = 265.08, P < 0.0001$, as was the Block effect, $F(9,60) = 5.67, P < 0.0001$, and the Group \times Block interaction, $F(9,60) = 5.15, P < 0.0001$. For animals with electrodes and freely moving eyes, there was a significant Group effect $F(1,60) = 125.21, P < 0.0001$, no significant Block effect $F(9,60) = 1.52, P > 0.25$, and no significant Group \times Block interaction $F(9,60) = 0.691, P > 0.25$. For animals with electrodes and the eye restrained, there was a significant Group effect $F(1,100) = 24.09, P < 0.0001$, no Block effect $F(9,100) = 1.42, P > 0.10$ and no Group \times Block interaction, $F(9,100) = 1.39, P > 0.10$.

With regard to sensitization, the effect was limited to the initial trials. As training continued, the group differences between animals with electrodes and those without was not significant. As noted above, paired animals with electrodes responded more to the CS at the outset of training than those without electrodes. A somewhat similar trend was observed for unpaired animals: unpaired animals with electrodes and the eye restrained made more responses during the first five CS presentations (mean probability 0.50, SD 0.35) than either the unpaired animals with electrodes and eye freely moving (mean 0.10, SD 0.12) or unoperated unpaired animals (mean .05, SD 0.1). Overall, however, analysis of variance conducted over the 10 five-trial blocks of acquisition revealed no group differences between animals with electrodes and those without: a comparison of animals with electrodes *versus* unoperated animals reveal no Group effect $F(1,60) = 0.631, P > 0.25$, a significant Block effect $F(9,60) = 4.15, P < 0.005$, and no Group \times Block interaction $F(9,60) = 1.63, P > 0.10$. Also, no significant Group, Trial, or Interaction effects ($P > 0.10$) were obtained for animals with electrodes and freely moving eyes versus those with electrodes and the eye restrained. An overall analysis of variance conducted over the 10 five-trial blocks for the three unpaired groups indicated no Group effect $F(2,90) = 1.37, P > 0.25$, no Block

effect $F(9,90) = 1.45, P > 0.05$, but a significant Group \times Block interaction $F(18,90) = 2.19, P < 0.01$. The significant interaction reflects the fact that two of the four animals in the unpaired group with electrodes and restrained eyes responded substantially during the first five CS presentations and that such responding decreased over the course of further unpaired training.

The major conclusion about the behavior of animals that had been trained with eyes restrained is that the performance in reacquisition is similar to the groups with freely moving eyes. Also, the unpaired controls with restrained eyes were capable of learning as shown in reacquisition, were not repressed due to unpaired pre-exposure, and had not fortuitously made a CS-US association. The acquisition performance of all paired groups was enhanced during reacquisition. For paired animals without electrodes, analysis of variance indicated significant Group effect $F(1,60) = 17.31, P < 0.0001$, a Block effect $F(9,60) = 6.42, P < 0.0001$, and a Group \times Block interaction $F(9,60) = 2.73, P < 0.01$. Analysis of the reacquisition performance of paired animals with electrodes and the eye free to move revealed a significant Group effect $F(1,60) = 12.64, P < 0.005$, no Block effect $F(9,60) = 0.676, P > 0.25$, and no Group \times Block interaction $F(9,60) = .676, P > .25$. A significant Group effect was also obtained in paired animals with electrodes and the eye restrained $F(1,60) = 96.50, P < 0.0001$. There was a Block effect $F(9,60) = 2.02, P < 0.05$, but no significant Group \times Block interaction $F(9,60) = 0.546, P > 0.25$.

As Figures 2, 4, and 5 suggest, the performance of unpaired animals was greatly enhanced when they received paired training. Analysis of variance of unpaired animals with no electrodes revealed a significant Group effect $F(1,60) = 79.34, P < 0.0001$, but no Block effect $F(9,60) = 1.02, P > 0.25$, or Group \times Block interaction $F(9,60) = 1.07, P > 0.25$. Analysis of unpaired animals with electrodes and the eye free to move indicated a significant Group effect $F(1,60) = 98.97, P < 0.0001$, Block effect $F(9,60) = 3.75, P < 0.005$, and Group \times Block interaction $F(9,60) = 2.66, P < 0.025$. Unpaired subjects with electrodes and the eye restrained (the eye was free to move during the reacquisition phase) also had a significant Group effect $F(1,60) = 93.28, P < 0.0001$, no Block effect $F(9,60) = 0.709, P > 0.25$, but a significant Group \times Block interaction $F(9,60) = P < 0.0001$.

Discussion

The major goal in this work was to determine the role of eye movement in classical conditioning of the withdrawal reflex. We wanted to determine, first, if eye movement is *necessary* for classical conditioning of the eye withdrawal; that is, whether any animals are capable of

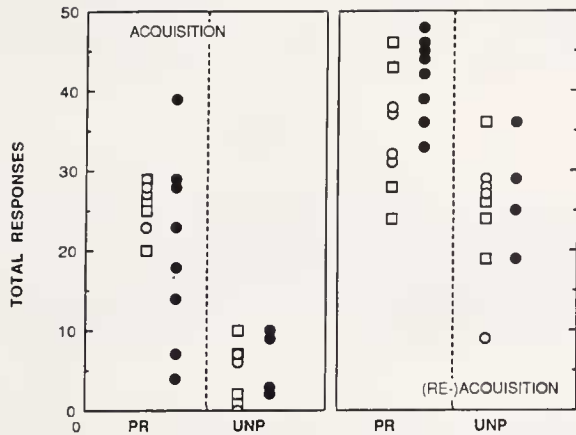


Figure 5. Total responses in acquisition and re-acquisition (50 trials). Data for animals with freely moving eyes are shown with open symbols and includes two subgroups: normal animals (circles) and animals with EMG electrodes (squares). Each experimental population, paired group (PR) and unpaired (UNP), includes 4 normals and 4 EMGs, although some data points overlap. Data for animals with restrained eyes during acquisition are shown as filled circles. There were eight animals in the PR groups and 8 in the UNP group, although some points overlap. Note that the PR group in ACQUISITION is given a second day of training in RE-ACQUISITION, but the group labeled UNP was a control only on the first day. In RE-ACQUISITION it was now presented with paired stimuli as described in the text.

learning when the eye is restrained. Our results show clearly that some animals can, in fact, learn with immobilized eyes. Having established that eye movement is not necessary, we next asked whether any animals ever used signals that arise from eye movement. Two of the eight animals with restrained eyes showed no normal acquisition of the EMG response. Was this because of experimental error, such as damage to the eye from implanting electrodes? Or did these two particular animals normally use a strategy of acquisition that required eye movement accounting for their poor performance when the eye was restrained? Although we cannot rule out an effect of eye movement on these animals, there is evidence that some learning took place even for these animals. When tested behaviorally, all animals performed like normals, particularly in re-acquisition. Also, small EMG bursts were evoked by CSs (but did not appear in the interval between stimuli). Thus, damage to the muscle might have non-specifically reduced the appearance of conditioned responses. Moreover, the eye withdrawal reflex, under normal conditions, proceeds without proprioceptive feedback (Burrows, 1967; Sandeman, 1967), although we do not know whether feedback can affect learning. In the optokinetic response, furthermore, motor output to the eye is driven by the difference in velocity of the eye and of the target (Horridge and Sandeman, 1964; Sandeman *et al.*, 1975; Erber and Sandeman, 1989). This motor output is normally overridden by the

eye withdrawal (Burrows and Horridge, 1968), but we cannot exclude the possibility that such a signal could be used by some crabs as part of a strategy in classical conditioning.

Comparison of classical conditioning and signalled avoidance

Our work supports the idea that classical conditioning of the eye withdrawal is simply dependent on the integration of the two sensory stimuli (vibration and air-puff). The results also bear on the study of this reflex in signalled avoidance, and the apparently paradoxical behavior of controls in that procedure. The problem may be summarized as follows. Signalled avoidance is designed as an operant procedure. If the animal makes a response during the presentation of the (signal) CS, the US is omitted, that is, the animal can avoid the aversive stimulus by its own behavior. However, animals that learn in an avoidance procedure may be undergoing a predominantly Pavlovian process; the procedure is identical to a classical conditioning experiment in which some USs have been omitted. Thus, the animal may learn during the CS-US pairings, but may not associate the occasional omission with its own behavior. In our study of signalled avoidance in the eye withdrawal in the crab, we found that animals learned well, but acquisition curves were essentially the same as those for animals in classical conditioning (Abramson *et al.*, 1988), suggesting a Pavlovian interpretation. The apparent paradox is that "yoked" controls presented with the same pattern of CSs and USs (some of which are now omitted) as the experimental animals, but independent of the responses they made, did not perform as well. Since these controls receive the same number of CSs and USs as the experimentals—the only difference is the contingency between stimuli and the animal's behavior—they should do as well (if the mechanism is truly Pavlovian). One of the following hypotheses could explain these seemingly paradoxical results.

One possibility is that learning in both the avoidance procedure and "classical conditioning" procedure is actually the same and substantially operant; reinforcement is provided by attenuation of the air-puff when the eye is retracted during the CR. If this is true, the yoke controls are behaving as per experimental design. The results presented here exclude this mechanism because animals can learn when their eye is retracted and there is no attenuation of the air-puff.

A second hypothesis is that avoidance learning is actually Pavlovian in mechanism; the yoke controls are giving erroneous data due to one of several possibilities. First, it is possible that yoke controls are actually subjected to different stimuli than experimentals as described by Woodward and Bitterman (1973). According

to this theory, there are actually two CSs: CS+ and CS-. These are compound stimuli composed of the vibration and some sensory information about whether the eye is up or down (for example, visual field). For experimentals, CS+ is (the state of eye-up) + vibration, which is predictably followed by the US; CS- is (eye-down) + vibration, predictably followed by an omission; these are randomized when vibration is presented to yoke controls. We have excluded this theory for classical conditioning and, therefore, for a Pavlovian interpretation of avoidance, by showing that animals can be conditioned with the eye restrained.

We favor an alternative explanation: that both classical conditioning and avoidance are Pavlovian in mechanism, but that the process involves two conditioned states, one of which has a higher probability of response than the other and is more resistant to extinction. Such a mechanism resembles the Markov chain model for conditioning (Theios and Brelsford, 1966). Experimental animals in avoidance, then, receive omissions at times when they are most resistant to extinction (high probability state), whereas for yokes, omissions are randomized. A similar explanation for experimental-yoke differences was proposed by Gormezano (1965) for the rabbit nictitating membrane.

Thus, the current work on classical conditioning allows us to exclude two of the possible explanations for signalled avoidance learning in the crab eye. We cannot, however, exclude the possibility that the mechanism of learning is actually different for the two procedures. Possibly the rates of acquisition for avoidance are the same as in classical conditioning because they share a common rate-determining step, probably at the output end of the behavior. For example, there may be a maximum rate of change in properties of the motor neuron. If this were so, the yoke controls would be performing as expected. At this point, we favor the Pavlovian interpretation. From the biological point of view, an all-or-none defensive reflex, such as eye withdrawal, probably does not require the subtle information about the effects of the behavior that an operant mechanism would impart.

In summary, EMGs recorded from muscle 19a of the eye can be used to study the acquisition of classical conditioning in animals with freely moving and immobilized eyes. Experiments using this method show that eye movement is not required for learning.

Acknowledgments

This work was supported in part by grant BNS-8819830 from the National Science Foundation, funds

from Scott, Sperry & Hanson, Inc., and funds from the Research Foundation of the State University of New York.

Literature Cited

- Abramson, C. I., P. M. Armstrong, R. A. Feinman, and R. D. Feinman. 1988. Signalled avoidance in the eye withdrawal reflex of the green crab. *J. Exp. Anal. Behav.* **50**: 483-492.
- Abramson, C. I., and R. D. Feinman. 1987. Operant punishment in the green crab, *Carcinus maenas*. *Behav. Neural Biol.* **48**: 259-277.
- Abramson, C. I., and R. D. Feinman. 1988. Classical conditioning of the eye withdrawal reflex in the green crab. *J. Neurosci.* **8**: 2907-2912.
- Appleton, T., and J. L. Wilkens. 1990. Habituation and sensitization and the effect of serotonin on the eyestalk withdrawal reflex of *Cancer magister*. *Comp. Biochem. Physiol. A* (in press).
- Burrows, M. 1967. Reflex withdrawal of the eyecup in the crab *Carcinus*. *Nature* **215**: 56-57.
- Burrows, M., and G. A. Horridge. 1968. Motoneuron discharges to the eyecup muscles of the crab *Carcinus*. *J. Exp. Biol.* **49**: 251-267.
- Erber, J., and D. C. Sandeman. 1989. The effect of serotonin and octopamine on the optokinetic response of the crab *Leptograpsus variegatus*. *J. Neurobiol.* **20**: 667-680.
- Forman, R., and D. Brumbley. 1980. An improved capacitative position transducer for biological systems. *J. Exp. Biol.* **88**: 399-402.
- Gormezano, I. 1965. Yoked comparisons of classical and instrumental conditioning of the eyelid response. Pp. 48-70 in *Classical Conditioning: A Symposium*. Appleton-Century-Crofts, New York.
- Horridge, G. A., and D. C. Sandeman. 1964. Nervous control of optokinetic responses in the crab *Carcinus*. *Proc. R. Soc. (B)*. **161**: 216-246.
- Miall, R. C., and C. J. Hereward. 1988. A simple miniature capacitative position transducer. *J. Exp. Biol.* **138**: 541-544.
- Moore, J. W., and I. Gormezano. 1961. Yoked comparisons of instrumental and classical eyelid conditioning. *J. Exp. Psych.* **62**: 552-559.
- Sandeman, D. C. 1964. Functional distinction between oculomotor and optic nerve in *Carcinus*. *Science* **201**: 302-303.
- Sandeman, D. C. 1967. Excitation and inhibition of the reflex withdrawal of the crab *Carcinus*. *J. Exp. Biol.* **46**: 475-485.
- Sandeman, D. C. 1968. A sensitive position measuring device for biological systems. *Comp. Biochem. Physiol.* **24**: 635-638.
- Sandeman, D. C. 1969a. Integrative properties of a reflex motoneuron in the brain of the crab *Carcinus maenas*. *Z. Vergl. Physiol.* **64**: 290-464.
- Sandeman, D. C. 1969b. The synaptic link between the sensory and motoneurons in the eye-withdrawal reflex of the crab. *J. Exp. Biol.* **50**: 87-98.
- Sandeman, D. C., J. Erber, and J. Kien. 1975. Optokinetic eye movements in the crab, *Carcinus maenas*. I. Eye torque. *J. Comp. Physiol.* **101**: 259-274.
- Theios, J., and J. W. Brelsford Jr. 1966. A Markov model for classical conditioning: applications to eye-blink conditioning in rabbits. *Psych. Rev.* **73**: 393-405.
- Woodward, W., and M. E. Bitterman. 1973. Pavlovian analysis of avoidance conditioning in the goldfish (*Carassius auratus*). *J. Comp. Physiol. Psychol.* **82**: 123-129.